J. Pharm. Pharmacol. 1992, 44: 244–249
Received June 4, 1991

A Possible Mechanism of Endothelium-dependent Relaxation Induced by Pirarubicin and Carbachol in Rat Isolated Aorta

SHIN-ICHI HIRANO, NAOKI AGATA, YUTAKA HARA, HIROSHI IGUCHI, MASATAKA SHIRAI, HIROSHI TONE AND NORIMOTO URAKAWA*

*Central Research Laboratories, Mercian Corp., Fujisawa 251, Japan and *Department of Veterinary Pharmacology, Nippon Veterinary and Zootechnical College, Musashino-Shi, Tokyo 180, Japan*

Abstract—The mechanism of endothelium-dependent relaxation induced by pirarubicin, (2″*R*)-4′-*O*-tetrahydropyranyladriamycin, THP, or carbachol was investigated in the rat isolated aorta. The relaxant effect of THP ($1{\cdot}5 \times 10^{-6}$–$4{\cdot}5 \times 10^{-5}$ M) or carbachol (10^{-8}–10^{-4} M) on the aorta with endothelium was decreased by lowering Ca^{2+} in the medium. The relaxation induced by THP was not inhibited by pretreatment with verapamil (10^{-6}–10^{-5} M), and that induced by carbachol was only partially inhibited. However, on replacement of all but 20 mM Na^+ with either Li^+ or choline, the THP- or carbachol-induced relaxation was inhibited. Furthermore, the relaxing effect of THP or carbachol was inhibited by pretreatment with amiloride (10^{-4}–3×10^{-4} M), with ouabain (10^{-4}–10^{-3} M), or with K^+-depletion. These results suggest that the THP- or carbachol-induced relaxation depending on endothelium was affected by modifying the calcium ion concentration, and that a Na^+–Ca^{2+} exchange process is involved.

Pirarubicin (2″*R*)-4′-*O*-tetrahydropyranyladriamycin, a derivative of doxorubicin (adriamycin), has been found to have a potent antitumour effect similar to doxorubicin itself, but with lower cardiotoxicity (Danchev et al 1979; Matsushita et al 1985). Pirarubicin has been conventionally abbreviated as THP (Majima 1982). THP has been shown to have a depressor effect in anaesthetized cats and rats (Tone et al 1986). We have reported that THP produced an endothelium-dependent relaxation in the rat isolated aorta (Hirano et al 1991a). Furthermore, in our preliminary study we reported that the THP-induced relaxation was probably mediated by endothelium-derived relaxing factor (EDRF) and that the tetrahydropyranyl group plays an important role in the THP-induced relaxation (Hirano et al 1991b). However, the mechanism of the endothelium-dependent relaxation induced by THP has been unclear, although it is known that cholinergic agents such as acetylcholine and carbachol cause a relaxation of vascular smooth muscle cells by releasing EDRF from the vascular endothelial cells (Furchgott & Zawadzki 1980; Murakami et al 1985; Nagase et al 1987). The mechanisms governing the release of EDRF have been investigated extensively and have, in particular, included the posible role of calcium ions. For instance, it has been shown that the calcium ionophore, A23187, produces an endothelium-dependent relaxation (Zawadzki et al 1980) and that acetylcholine activates muscarinic receptors (M_2) which results in an increased influx of Ca^{2+} into endothelial cells (Long & Stone 1985; Peach et al 1987). However, the route of the entry of the extracellular Ca^{2+} into endothelial cells causing EDRF release remains controversial. While some studies have demonstrated that the Ca^{2+} influx occurred through the membrane via voltage-dependent calcium channels (Singer & Peach 1982; Williams et al 1987), others reported a lack of involvement of these channels (Miller et al 1985; Winquist et al 1985; Jayakody et al 1987) and the possible involvement of the Na^+–Ca^+ exchange system (Winquist et al 1985; Shoeffter & Miller 1986). The objective of the present study was to investigate the role of cellular Ca^{2+} entering via the Na^+–Ca^{2+} exchange system for the endothelium-dependent relaxations produced by either THP or carbachol.

Correspondence: S.-I. Hirano, Central Research Laboratories, Mercian Corporation, 9-1 Johnan 4 Chome, Fujisawa 251, Japan.

Materials and Methods

Rat aortic strips

Sprague-Dawley rats (male, 250–300 g) were killed by a blow on the head and exsanguinated. The thoracic aorta was removed and placed in physiological saline solution (PSS). Each thoracic aorta was carefully cleaned of surrounding connective tissue and cut into several helical strips, 2–3 mm wide and 8–10 mm long. Each muscle strip with endothelium was attached to a holder under a resting tension of 0·5 g and equilibrated for 60 min in a 10 mL organ bath. Muscle contractions were recorded isometrically with a force transducer (TB-611T, Nihon Kohden, Tokyo, Japan) connected to a multipurpose polygraph (RM-6000, Nihon Kohden). Each preparation was checked to confirm that 10^{-6} M carbachol induced an almost complete (more than 80%) relaxation of the 10^{-7} M noradrenaline-induced contraction in order to determine the functional integrity of the endothelium.

In experiments with low- or high-Ca^{2+} solutions, tissues were initially washed in Ca^{2+}-free PSS for 10 min, then incubated in 0·2, 0·4, 0·5, 0·8, 2·0 or 10·0 mM Ca^{2+} PSS for 15 min, respectively. Subsequently, 10^{-7} M noradrenaline was used to precontract the aorta in a medium containing each Ca^{2+} concentration; THP ($1{\cdot}5 \times 10^{-6}$–$4{\cdot}5 \times 10^{-5}$ M) or carbachol (10^{-8}–10^{-4} M) was cumulatively applied to the medium.

Solutions

Normal PSS contained (mM): NaCl 118·3, KCl 4·7, $CaCl_2$ 2·0, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, KH_2PO_4 1·2, calcium EDTA

0·026 and glucose 11·1. The solution was maintained at 37°C and continuously gassed with 95% O_2–5% CO_2 (pH 7·4). The low- or high-Ca^{2+} solutions were prepared by a decrease or increase of $CaCl_2$ from the normal PSS. The Ca^{2+}-free solution was made by omitting $CaCl_2$ and calcium EDTA from the normal PSS and adding 0·1 mM disodium EGTA. The low-Na^+ solution contained (mM): NaCl 14·0, KCl 4·7, LiCl or choline chloride 104·3, $NaHCO_3$ 6·0, KH_2PO_4 1·2, $CaCl_2$ 2·5, calcium EDTA 0·026 and glucose 11·1, equilibrated to pH 7·1 by aeration with 95% O_2–5% CO_2. The K^+-free solution was prepared by omitting KCl from the normal PSS and by replacing the KH_2PO_4 with NaH_2PO_4.

Drugs

THP was synthesized from daunorubicin (daunomycin) in our laboratory. THP was dissolved in deionized water. Other drugs used in this study were (−)-noradrenaline bitartrate (Wako, Osako, Japan), carbamylcholine chloride (carbachol, Wako), verapamil hydrochloride (Eizai), atropine sulphate (Tanabe, Osaka, Japan), amiloride hydrochloride (Sigma), (−)-ouabain octahydrate (Aldrich, Milwaukee MI), lithium chloride (Wako), and choline chloride (Wako). These drugs were dissolved in deionized water, with the exception of amiloride and ouabain which were dissolved in PSS.

Statistical comparison

Results of the experiments were expressed as mean ± s.e.m. The data were analysed by Student's unpaired *t*-test and $P < 0.05$ was defined as significant.

Results

Effects of external calcium and of verapamil

As shown in Figs 1B and 2A, the relaxation caused by THP (4.5×10^{-6}–4.5×10^{-5} M) was inhibited in 0·4 mM Ca^{2+} PSS. The relaxation by THP (4.5×10^{-6} M) was also inhibited in 0·8 mM Ca^{2+} PSS. However, in 10·0 mM Ca^{2+} PSS the THP-induced relaxation was not inhibited (Fig. 2A). As shown in Figs 1D and 2B, the noradrenaline-induced contraction was suppressed in 0·2 mM Ca^{2+} PSS and the relaxation caused by carbachol (10^{-7}–10^{-4} M) was inhibited in this calcium concentration. The relaxation by carbachol (10^{-6}–10^{-4} M) was also inhibited in 0·5 mM Ca^{2+} PSS, but not in 0·8 or 10·0 mM Ca^{2+} PSS (Fig. 2B). The relationship between external calcium concentration (0·4–2·0 mM) and relative relaxation of THP (1.5×10^{-5} M) was almost linear. Also, the relative relaxation of carbachol (10^{-5} M) was almost linear over the range 0·2–2·0 mM calcium. The curve of THP is slightly steeper than that of carbachol (Fig. 3).

As shown in Fig. 4A, pretreatment with verapamil (10^{-6}–10^{-5} M) for 30 min had no effect on the relaxation caused by THP (1.5×10^{-6}–4.5×10^{-5} M). Carbachol-induced relaxation at concentrations ranging from 10^{-8} to 10^{-6} M was partially inhibited by verapamil (10^{-6}–10^{-5} M). The relaxation caused by carbachol (3×10^{-6}–10^{-5} M) was slightly inhibited by 10^{-6} M verapamil (Fig. 4B).

Although the THP- or carbachol-induced relaxation was modified by the external calcium concentration (0·4–2·0 mM), the THP-induced relaxation was not inhibited by the

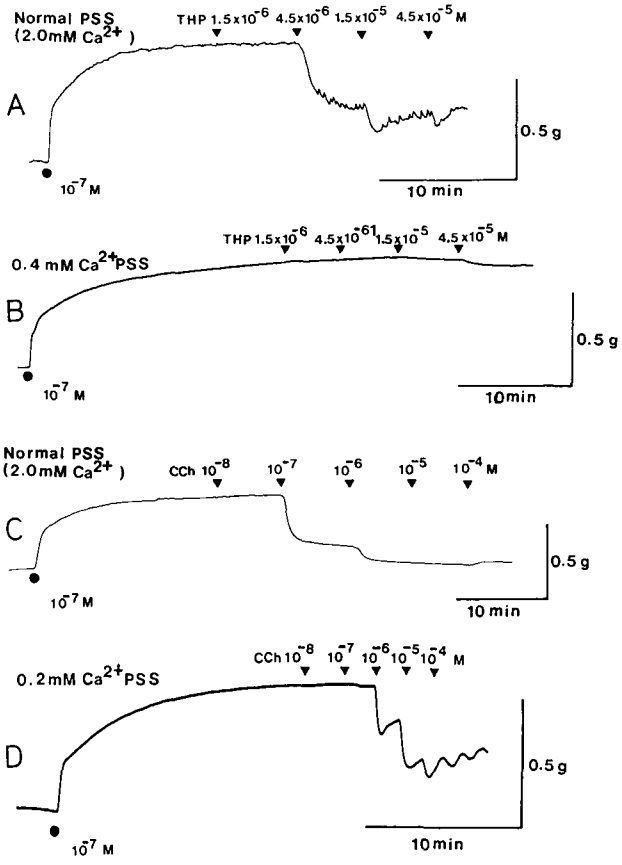

FIG. 1. Typical recordings of the THP- or carbachol-induced endothelium-dependent relaxation of the rat thoracic aorta in a normal- or low-Ca^{2+} physiological saline solution (PSS). THP or carbachol was applied cumulatively in the organ bath at concentrations from 1.5×10^{-6} to 4.5×10^{-6} M or from 10^{-8} to 10^{-4} M, respectively. A: THP-induced relaxation in normal PSS. B: THP-induced relaxation in 0·4 mM Ca^{2+} PSS. C: carbachol-induced relaxation in normal PSS. D: carbachol-induced relaxation in 0·2 mM Ca^{2+} PSS.

Ca^{2+} channel blocker, verapamil. The carbachol-induced relaxation was only partially inhibited.

Effect of amiloride

Experiments were also performed using amiloride, an antagonist of the Na^+–Ca^{2+} exchange system. The relaxation caused by THP (1.5×10^{-6}–4.5×10^{-5} M) was not inhibited by pretreatment with amiloride (10^{-4} M) for 30 min. In contrast, the relaxation caused by THP (1.5×10^{-5} and 4.5×10^{-5} M) was inhibited by pretreatment with 3×10^{-4} M amiloride (Fig. 5A). The relaxation caused by carbachol (3×10^{-8}–10^{-5} M) was also inhibited by pretreatment with 10^{-4} or 3×10^{-4} M amiloride, and the relaxation by carbachol (3×10^{-5} and 10^{-4} M) was inhibited by 3×10^{-4} M amiloride (Fig. 5B).

Effect of replacement of external Na^+ by Li^+ or choline

In an experiment with low-Na^+ solution, tissues were incubated in normal PSS or in a PSS where all but 20 mM Na^+ was replaced either by LiCl or by choline chloride (containing 10^{-6} M atropine sulphate) for 90 min. THP (1.5×10^{-6}–4.5×10^{-5} M) or carbachol (10^{-8}–10^{-4} M) was

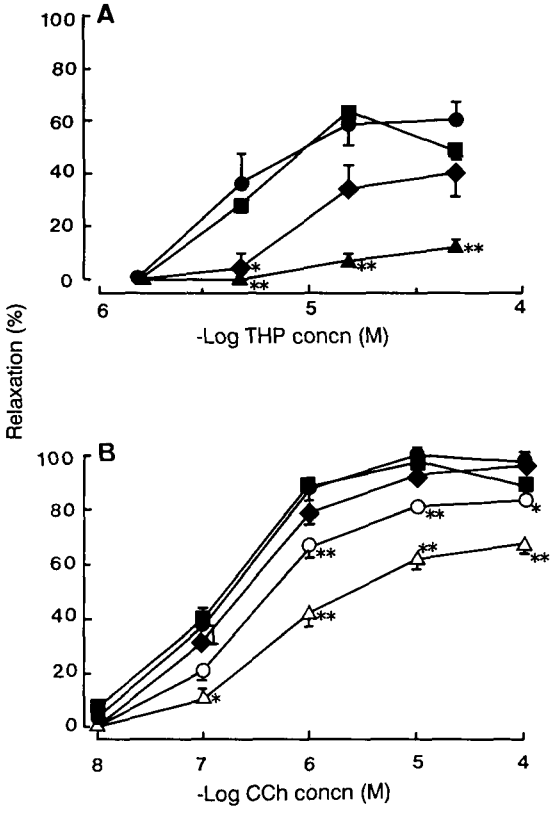

FIG. 2. Effects of external calcium on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. Experiments were performed as shown in Fig. 1. Data points are mean ± s.e.m. of 5 to 8 experiments. $^{**}P<0.01$ and $^{*}P<0.05$, compared with control ■ 10·0, ● 2·0, ◆ 0·8, ○ 0·5, ▲ 0·4 and △ 0·2 mM Ca^{2+}.

cumulatively applied after 10^{-7} M noradrenaline had induced a contraction. In the presence of LiCl, the relaxation caused by THP (4.5×10^{-6} and 1.5×10^{-5} M) or carbachol (3×10^{-8}–10^{-4} M) was inhibited (Fig. 6A, B). In the presence of choline, the relaxation caused by THP (1.5×10^{-5} and 4.5×10^{-5} M) was also inhibited (Fig. 6A).

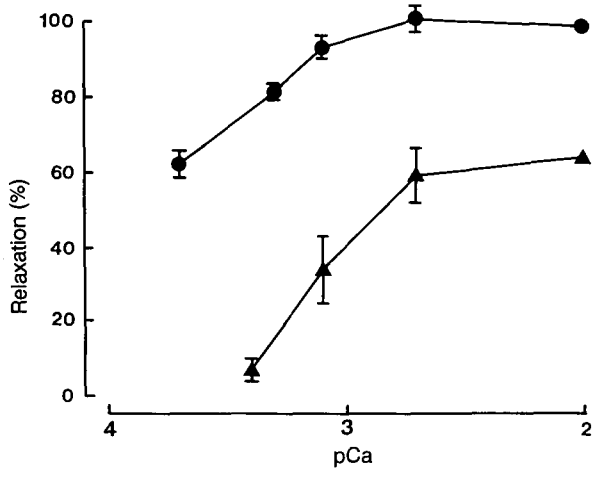

FIG. 3. Relationship between external calcium concentration and relative relaxation of THP (1.50×10^{-5} M, ▲) or carbachol (10^{-5} M, ●). Experiments were performed as shown in Fig. 1. Data points are mean ± s.e.m. of 5 to 8 experiments.

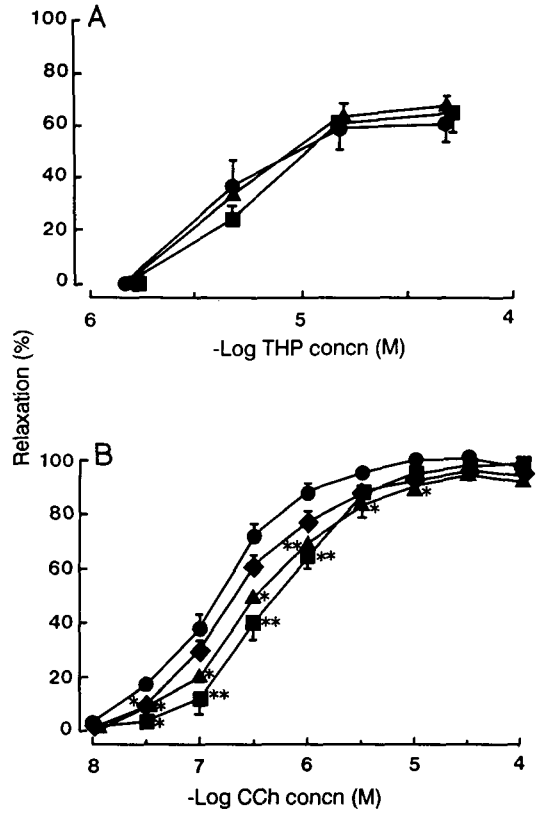

FIG. 4. Effects of pretreatment with verapamil (10^{-7} ◆, 10^{-6} ▲, 10^{-5} M ■) on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. THP or carbachol was applied cumulatively in the organ bath at concentrations from 1.5×10^{-6} to 4.5×10^{-5} M or from 10^{-8} to 10^{-4} M, respectively. Data points are mean ± s.e.m. of 4 to 8 experiments. $^{**}P<0.01$ and $^{*}P<0.05$ compared with control (●).

Effects of ouabain and K^+-depletion

The effects of a pretreatment with ouabain (10^{-4}–10^{-3} M) for 60 min on either the THP- or carbachol-induced relaxation are shown in Fig. 7A and B, respectively. Ouabain (10^{-4} M) slightly inhibited the relaxation caused by carbachol (10^{-5}–10^{-4} M), but not that by THP (1.5×10^{-6} –4.5×10^{-5} M). Ouabain at concentrations of 5×10^{-4} and 10^{-3} M caused an increase in basal tension which stabilized within 60 min, and the contractile response to noradrenaline (10^{-7} M) decreased (data not shown). Even under these conditions, the relaxation caused by THP (1.5×10^{-5} and 4.5×10^{-5}) or carbachol (3×10^{-8}–10^{-4} M) was markedly inhibited by ouabain.

In experiments involving K^+-depletion, tissues were incubated in normal or K^+-free PSS for 60 min, and then THP (1.5×10^{-6}–4.5×10^{-5} M) or carbachol (10^{-8}–10^{-4} M) was cumulatively applied to the 10^{-7} M noradrenaline-induced contraction. After incubation with K^+-free PSS for 60 min, an increase in basal tension was observed (data not shown). The relaxation caused by THP (4.5×10^{-6}–4.5×10^{-5} M) or carbachol (3×10^{-8}–10^{-4} M) during the noradrenaline-induced contraction was markedly inhibited by K^+-depletion, as shown in Fig. 8.

Discussion

Ca^{2+} plays an essential role on the cellular mechanisms underlying EDRF generation or release (Long & Stone 1985;

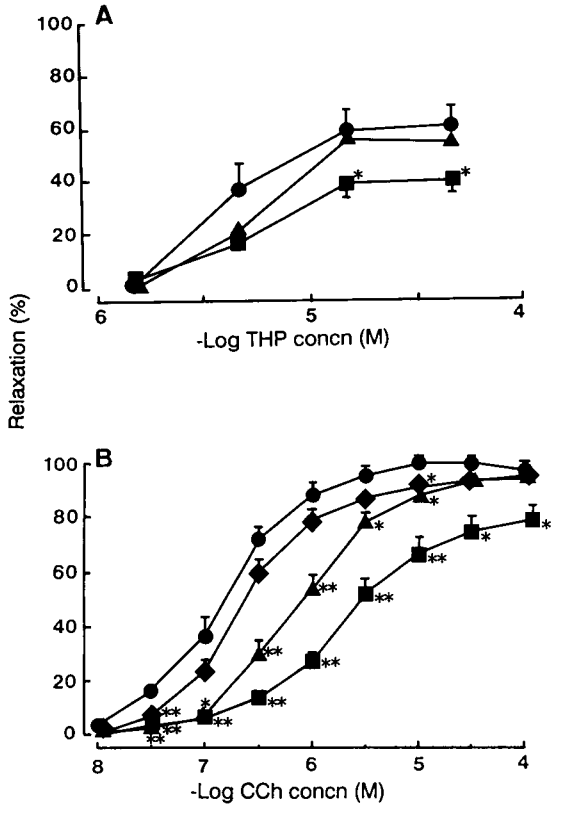

FIG. 5. Effects of pretreatment with amiloride (10^{-5} ◆, 10^{-4} ▲, 3×10^{-4} M ■) on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. THP or carbachol was applied cumulatively in the organ bath at concentrations from $1{\cdot}5 \times 10^{-6}$ to $4{\cdot}5 \times 10^{-5}$ M or from 10^{-8} to 10^{-4} M, respectively. Data points are mean ± s.e.m. of 5 to 8 experiments. $^{**}P < 0{\cdot}01$ and $^{*}P < 0{\cdot}05$ compared with control (●).

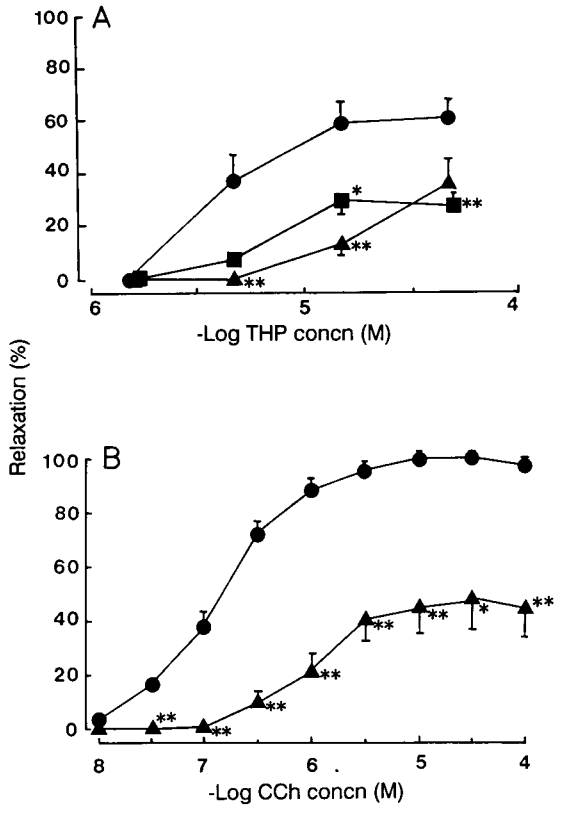

FIG. 6. Effect of replacing all except 20 mM Na^+ by Li^+ (▲) or choline (■) on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. THP or carbachol was applied cumulatively in the organ bath at concentrations from $1{\cdot}5 \times 10^{-6}$ to $4{\cdot}5 \times 10^{-5}$ M or from 10^{-8} to 10^{-4} M, respectively. Data points are mean ± s.e.m. of 4 to 5 experiments. $^{**}P < 0{\cdot}01$ and $^{*}P < 0{\cdot}05$ compared with control (●).

Peach et al 1987). It has been reported that intracellular Ca^{2+} ($[Ca^{2+}]_i$) is increased by several agonists (ATP, bradykinin, histamine, thrombin, and the Ca^{2+} ionophore, A23187) known to induce both endothelium-dependent relaxation and EDRF release in vascular smooth muscle cells (Lückhoff & Busse 1986; Griffith et al 1986; Colden-Stanfield et al 1987; Jaffe et al 1987; Peach et al 1987; Pirotton et al 1987; Brock & Capasso 1988). Acetylcholine has been reported to increase $[Ca^{2+}]_i$ in endothelial cells (Gorden & Martin 1983; Peach et al 1985; Winquist et al 1985; Lückhoff & Busse 1986; Shoeffter & Miller 1986; Danthuluri et al 1988; Oleson et al 1988). However, the route of entry of extracellular Ca^{2+} responsible for the release of EDRF is uncertain. Singer & Peach (1982) showed that the calcium channel blockers, nifedipine and verapamil, inhibited methacholine- and A23187-induced endothelium-dependent relaxation. Other investigators have failed to block the endothelium-dependent relaxation to acetylcholine with the calcium antagonists, verapamil and nifedipine (Winquist et al 1985).

We have previously reported that THP produced an endothelium-dependent relaxation in rat isolated aorta (Hirano et al 1991a), which was probably mediated by EDRF (Hirano et al 1991b). We have attempted to clarify the role of Ca^{2+} for the endothelium-dependent relaxation induced by THP. The THP-induced relaxation was modified by external Ca^{2+}, but insensitive to the Ca^{2+} channel blocker. The carbachol-induced relaxation was modified by external Ca^{2+}, and was only partially inhibited by verapamil. The THP-induced relaxation was more affected than the carbachol-induced relaxation by external Ca^{2+} concentration. From these data, it is suggested that the increase in $[Ca^{2+}]_i$, via verapamil-resistant pathways is essential in the THP- or carbachol-induced relaxation.

Before the essential role of endothelium in the acetylcholine-induced relaxation of the vascular smooth muscle had been established, De Mey & Vanhoutte (1980) proposed that the acetylcholine response might be involved in the Na^+–Ca^{2+} exchange system. Winquist et al (1985) and Shoeffter & Miller (1986) reported data concerning the possible involvement of the Na^+–Ca^{2+} exchange mechanism in EDRF-synthesis or release. That is, the EDRF-mediated vasorelaxation induced by acetylcholine and other EDRF-dependent vasodilators was inhibited by removal of extracellular sodium, resulting in inhibition of the Na^+–Ca^+ exchange mechanism, by addition of amiloride or dichlorobenzamil, two inhibitors of the Na^+–Ca^{2+} exchange mechanism, or by the presence of ouabain, an inhibitor of Na^+–K^+-ATPase, which is coupled to the Na^+–Ca^{2+} exchange mechanism (De Mey & Vanhoutte 1980; Winquist et al 1985; Shoeffter & Miller 1986). In the present experiments, the THP-induced

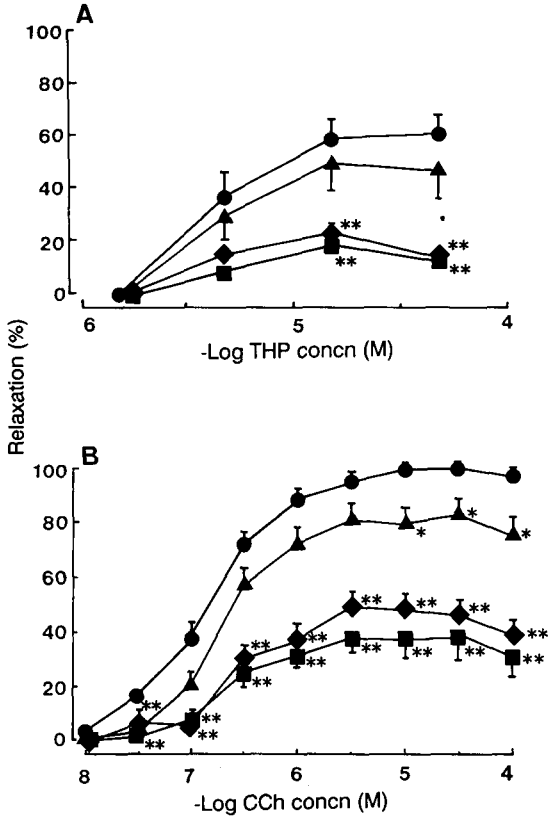

FIG. 7. Effects of pretreatment with ouabain (10^{-4} ▲, 5×10^{-4} ◆, 10^{-3} M ■) on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. THP or carbachol was applied cumulatively in the organ bath at concentrations from $1{\cdot}5 \times 10^{-6}$ to $4{\cdot}5 \times 10^{-5}$ M or from 10^{-8} to 10^{-4} M, respectively. Data points are mean ± s.e.m. of 4 to 5 experiments. $^{**}P < 0{\cdot}01$ and $^{*}P < 0{\cdot}05$ compared with control (●).

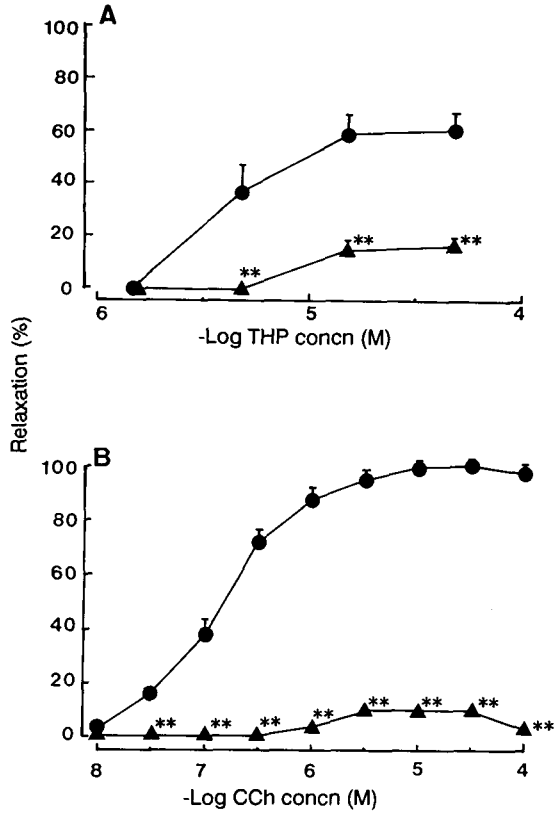

FIG. 8. Effect of K^+-depletion (▲) on the concentration-relaxation curves to THP (A) or carbachol (B) in noradrenaline-precontracted thoracic rat aorta with endothelium. THP or carbachol was applied cumulatively in the organ bath at concentrations from $1{\cdot}5 \times 10^{-6}$ to $4{\cdot}5 \times 10^{-5}$ M or from 10^{-8} to 10^{-4} M, respectively. Data points are mean ± s.e.m. of 4 to 5 experiments. $^{**}P < 0{\cdot}01$ compared with control (●).

relaxation was inhibited by replacing all but 20 mM Na^+ of the PSS by Li^+, or choline. The carbachol-induced relaxation was also inhibited by replacing all but 20 mM Na^+ by Li^+. Further, the THP- or carbachol-induced relaxation was inhibited by pretreatment with amiloride, with ouabain, or with K^+-depletion. K^+-Depletion as well as ouabain treatment is known to inhibit the Na^+-K^+-ATPase coupling Na^+-Ca^{2+} exchange system. These results suggest that the Na^+-Ca^{2+} exchange system is important for the EDRF-dependent relaxant effect of THP or carbachol, which is consistent with the finding that carbachol-increased $[Ca^{2+}]_i$ in the endothelial cells was less sensitive to the Ca^{2+} antagonist in rat aortic strips, simultaneously measured muscle tension and endothelial $[Ca^{2+}]_i$ using fura-2 (Sato et al 1990), and also that increases in intracellular Na^+ can lead to a rise in free cytoplasmic Ca^{2+} concentration either by facilitation of Ca^{2+} influx via a Na^+-Ca^{2+} exchange system at the plasma membrane (Rubanyi 1986) or via Na^+-induced Ca^{2+} release from intracellular binding sites (Van Breemen et al 1979).

References

Brock, T. A., Capasso, E. L. (1988) Thrombin and histamine activate phospholipase C in human endothelial cells via a phorbol ester-sensitive pathway. J. Cell. Physiol. 136: 54–62

Colden-Stanfield, M., Schilling, W. P., Ritchie, A. K., Eskin, S. G., Navarro, L. T., Kunze, D. L. (1987) Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. Circ. Res. 61: 632–640

Danthuluri, N. R., Cybulsky, M. I., Brock T. A. (1988) ACh-induced calcium transients in primary cultures of rabbit aortic endothelial cells. Am. J. Physiol. 255: H1533–H1549

De Mey, J. G., Vanhoutte, P. M. (1980) Interaction between Na^+, K^+ exchanges and the direct inhibitory effect of acetylcholine in canine femoral arteries. Circ. Res. 46: 826–836

Danchev, D., Paintrand, M., Hayat, M., Bourut, C., Mathè, G. (1979) Low heart and skin toxicity of a tetrahydropyranyl derivative of adriamycin (THP-ADM) as observed by electron and light microscopy. J. Antibiotics 32: 1085–1086

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Gorden, J. L., Martin, W. (1983) Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of ^{86}Rb efflux from isolated endothelial cells. Br. J. Pharmacol. 79: 531–541

Griffith, T. M., Edwards, D. H., Newby, A. C., Lewis, M. J., Henderson, A. H. (1986) Production of endothelium-derived relaxing factor is dependent on oxidative phosphorylation and extracellular calcium. Cardiovasc. Res. 20: 7–12

Hirano, S., Agata, N., Hara, U., Iguchi, H., Shirai, M., Tone, H., Urakawa, N. (1991a) Effects of pirarubicin, an antitumor antibiotic, on the cardiovascular system. Cancer Chemother. Pharmacol. 28: 266–272

Hirano, S., Agata, N., Hara, Y., Shirai, M., Urakawa, N. (1991b)

The property of pirarubicin-induced endothelium-dependent relaxation in vascular smooth muscle. Jpn. J. Pharmacol. 55 (Suppl. I): 161P

Jaffe, E. A., Grulich, J., Weksler, B. B., Hampel, G., Watanabe, K. (1987) Correlation between thrombin-induced prostacycline production and inositol trisphosphate and cytosolic free calcium levels in cultured human endothelial cells. J. Biol. Chem. 262: 8557–8565

Jayakody, R. L., Kappagoda, C. T., Senaratne, M. P. J., Sreeharan, N. (1987) Absence of effect of calcium antagonists on endothelium-dependent relaxation in rabbit aorta. Br. J. Pharmacol. 91: 155–164

Long, C. J., Stone, T. W. (1985) The release of endothelium-dependent relaxant factor is calcium dependent. Blood Vessels 22: 205–208

Lückhoff, A., Busse, R. (1986) Increased free calcium in endothelial cells under stimulation with adenine nucleotides. J. Cell. Physiol. 126: 414–420

Majima, H. (1982) Preliminary phase I study of 4′-O-tetrahydropranyl-adriamycin hydrochloride (THP). Proceedings of 13th International Cancer Congress. Seattle, Washington, p. 405

Matsushita, Y., Kumagai, H., Yoshimoto, A., Tone, H., Ishikura, T. (1985) Antitumor activities of (2″*R*)-4′-O-tetrahydropyranyladriamycin (THP) and its combination with other antitumor agents on murine tumors. J. Antibiotics 38: 1408–1419

Miller, R. C., Schoeffter, P., Stoclet, J. C. (1985) Insensitivity of calcium-dependent endothelial stimulation in rat isolated aorta to the calcium entry blocker, flunarizine. Br. J. Pharmacol. 85: 481–487

Murakami, K., Karaki, H., Urakawa, N. (1985) Role of endothelium in the contractions induced by norepinephrine and clonidine in rat aorta. Jpn. J. Pharmacol. 39: 357–364

Nagase, H., Karaki, H., Urakawa, N. (1987) Palytoxin-induced endothelium-dependent relaxation in the isolated rat aorta. Naunyn Schmiedebergs Arch. Pharmacol. 335: 575–579

Oleson, S. P., Davies, P. F., Clapham, D. E. (1988) Muscarinic-activated K^+ current in bovine aortic endothelial cells. Circ. Res. 62: 1059–1064

Peach, M. J., Singer, H. A., Loeb, A. L. (1985) Mechanisms of endothelium-dependent vascular smooth muscle relaxation. Biochem. Pharmacol. 34: 1867–1874

Peach, M. J., Singer, H. A., Izzo, N. J., Loeb, A. L. (1987) Role of calcium in endothelium-dependent relaxation of arterial smooth muscle. Am. J. Cardiol. 59: 35A–43A

Pirotton, S., Raspe, E., Demolle, D., Erneux, C., Boeynaems, J. M. (1987) Involvement of inositol 1,4,5,-triphosphate and calcium in the action of adenine nucleotides on aortic endothelial cells. J. Biol. Chem. 262: 17461–17466

Rubanyi, G. M. (1986) Potassium-induced release of endothelium-derived relaxing factor. Circulation 74: 414

Sato, K., Ozaki, H., Karaki, H. (1990) Differential effects of carbachol on cytosolic calcium levels in vascular endothelium and smooth muscle. J. Pharmacol. Exp. Ther. 255: 114–119

Shoeffter, P., Miller, R. C. (1986) Role of sodium-calcium exchange and effects of calcium entry blockers on endothelial-mediated responses in rat isolated aorta. Mol. Pharmacol. 30: 53–57

Singer, H. A., Peach, M. J. (1982) Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. Hypertension 4 (Suppl. II): 19–25

Tone, H., Kiyosaki, T., Nishimori, T., Kobayashi, F., Nishimura, K., Morino, H., Tsuchiyama, M. (1986) General pharmacology of (2″R)-4′-O-tetrahydropyranyladriamycin, a new antitumor antibiotic. Jpn. J. Antibiotics 39: 526–546 (in Japanese)

Van Breemen, C., Aaronson, P., Loutzenhiser, R. (1979) Sodium-calcium interactions in mammalian smooth muscle. Pharmacol. Rev. 30: 167–208

Williams, J. S., Baik, Y. H., Koch, W. J., Schwartz, A. (1987) A possible role for the endothelium in porcine coronary smooth muscle responses to dihydropyridine calcium channel modulators. J. Pharmacol. Exp. Ther. 241: 379–386

Winquist, R. J., Bunting, P. B., Schofield, T. L. (1985) Blockade of endothelium-dependent relaxation by the amiloride analog dichlorobenzamil: possible role of Na^+/Ca^{2+} exchange in the release of endothelium-derived relaxant factor. Ibid. 235: 644–650

Zawadzki, J. V., Cherry, P. D., Furchgott, R. F. (1980) Comparison of endothelium-dependent relaxation of rabbit aorta by A23187 and by acetylcholine. Pharmacologist 22: 271